

## SUPPLEMENTAL AMENDMENT

Serial Number: 09/689,136

Filing Date: October 12, 2000

Title: COMPOUNDS AND METHODS TO ENHANCE rAAV TRANSDUCTION

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In the Claims

Please amend the claims as follows:

1. (Currently Amended) A method to identify an agent that enhances adeno-associated virus (AAV) transduction of a mammalian cell comprising:
  - a) providing a mammalian cell contacted with an agent and AAV; and
  - b) identifying an agent that when contacted with the mammalian cell enhances AAV transduction after viral binding to the membrane of the contacted mammalian cell and before second strand synthesis which yields an expressible form of the viral genome, wherein the agent which is identified enhances AAV transport to the nucleus.

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2. (Previously Presented) The method of claim 1 or 87 wherein the cell is a mammalian lung cell.
3. (Previously Presented) The method of claim 1 or 87 wherein the cell is a mammalian liver cell.
4. (Previously Presented) The method of claim 1 or 87 wherein the cell is a human cell, canine cell, murine cell, rat cell or rabbit cell.
5. (Canceled)
6. (Currently Amended) The method of claim 1 or 87 wherein the mammalian cell is contacted with an agent that enhances endosomal processing.
7. (Currently Amended) The method of claim 1 or 87 wherein the mammalian cell is contacted with an agent that is an endosomal protease inhibitor.
8. (Currently Amended) The method of claim 7 wherein the mammalian cell is contacted with an agent that is a cysteine protease inhibitor.

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9. (Currently Amended) The method of claim 1 or 87 wherein the mammalian cell is contacted with an agent that is a peptide or analog thereof.
10. (Previously Presented) The method of claim 1 or 87 wherein the virus is recombinant AAV.
11. (Original) The method of claim 10 wherein the recombinant virus encodes a therapeutic peptide or polypeptide.
12. (Previously Presented) The method of claim 10 wherein the recombinant virus comprises a marker gene that is detectable or selectable.
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- 13-28. (Cancelled)
29. (Currently Amended) The method of claim 1 or 87 wherein the mammalian cell is contacted with an agent that is a compound of formula (I):  $R_1-A-(B)_n-C$  wherein  $R_1$  is an N-terminal amino acid blocking group; each A and B is independently an amino acid; C is an amino acid wherein the terminal carboxy group has been replaced by a formyl (CHO) group; and n is 0, 1, 2, or 3; or a pharmaceutically acceptable salt thereof.
30. (Original) The method of claim 29 wherein  $R_1$  is  $(C_1-C_{10})$ alkanoyl.
31. (Original) The method of claim 29 wherein  $R_1$  is acetyl or benzyloxycarbonyl.
32. (Original) The method of claim 29 wherein each A and B is independently alanine, arginine, glycine, isoleucine, leucine, valine, nor-leucine or nor-valine.
33. (Original) The method of claim 29 wherein each A and B is isoleucine.

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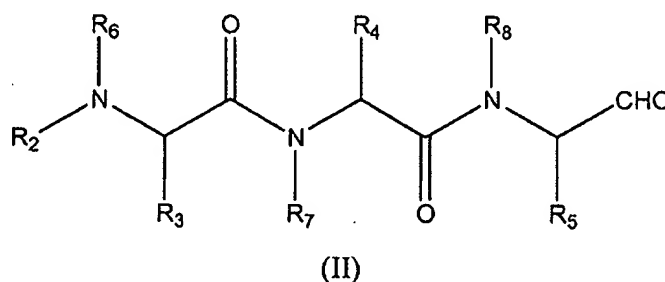
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34. (Original) The method of claim 29 wherein C is alanine, arginine, glycine, isoleucine, leucine, valine, nor-leucine or nor-valine, wherein the terminal carboxy group has been replaced by a formyl (CHO) group.
35. (Original) The method of claim 29 wherein C is nor-leucine or nor-valine, wherein the terminal carboxy group has been replaced by a formyl (CHO) group.
36. (Currently Amended) The method of claim 29 wherein R<sub>1</sub> is (C<sub>1</sub>-C<sub>10</sub>)alkanoyl or benzyloxycarbonyl; A and B are each isoleucine; C is nor-leucine or nor-valine, wherein the terminal carboxy group has been replaced by a formyl (CHO) group; and n is 1.
37. (Currently Amended) The method of claim 1 or 87 wherein the mammalian cell is contacted with an agent that is a compound of formula (II):



wherein

R<sub>2</sub> is an N-terminal amino acid blocking group;

R<sub>3</sub>, R<sub>4</sub>, and R<sub>5</sub> are each independently hydrogen, (C<sub>1</sub>-C<sub>10</sub>)alkyl, aryl or aryl(C<sub>1</sub>-C<sub>10</sub>)alkyl;

and

R<sub>6</sub>, R<sub>7</sub>, and R<sub>8</sub> are each independently hydrogen, (C<sub>1</sub>-C<sub>10</sub>)alkyl, aryl or aryl(C<sub>1</sub>-C<sub>10</sub>)alkyl; or a pharmaceutically acceptable salt thereof.

38. (Withdrawn) The method of claim 37 wherein R<sub>2</sub> is (C<sub>1</sub>-C<sub>10</sub>)alkanoyl.
39. (Withdrawn) The method of claim 37 wherein R<sub>2</sub> is acetyl or benzyloxycarbonyl.
40. (Withdrawn) The method of claim 37 wherein R<sub>3</sub> is hydrogen or (C<sub>1</sub>-C<sub>10</sub>)alkyl.

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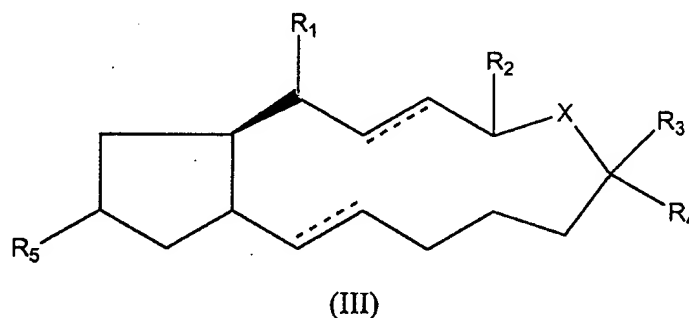
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41. (Withdrawn) The method of claim 37 wherein  $R_3$  is 2-methylpropyl.
42. (Withdrawn) The method of claim 37 wherein  $R_4$  is hydrogen or  $(C_1-C_{10})$ alkyl.
43. (Withdrawn) The method of claim 37 wherein  $R_4$  is 2-methylpropyl.
44. (Withdrawn) The method of claim 37 wherein  $R_5$  is hydrogen or  $(C_1-C_{10})$ alkyl.
45. (Withdrawn) The method of claim 37 wherein  $R_5$  is butyl or propyl.
46. (Withdrawn) The method of claim 37 wherein  $R_2$  is acetyl or benzyloxycarbonyl;  $R_3$  and  $R_4$  are each 2-methylpropyl;  $R_5$  is butyl or propyl; and  $R_6$ ,  $R_7$ , and  $R_8$  are each independently hydrogen.
47. (Currently Amended) The method of claim 1 or 87 wherein the mammalian cell is contacted with an agent that is a compound of formula (III):



wherein

$R_1$  is H, halogen,  $(C_1-C_{10})$ alkyl,  $(C_1-C_{10})$ alkenyl,  $(C_1-C_{10})$ alkynyl,  $(C_1-C_{10})$ alkoxy,  $(C_1-C_{10})$ alkanoyl,  $(=O)$ ,  $(=S)$ , OH, SR, CN,  $NO_2$ , or trifluoromethyl or  ~~$(C_1-C_{10})$ alkoxy~~, wherein any alkyl, alkenyl, alkynyl, alkoxy or alkanoyl may optionally be substituted with one or more halogen, OH, SH, CN,  $NO_2$ , trifluoromethyl, NRR or SR, wherein each R is independently H or  $(C_1-C_{10})$ alkyl;

$R_2$  is  $(=O)$  or  $(=S)$ ;

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R<sub>3</sub> is H, (C<sub>1</sub>-C<sub>10</sub>)alkyl, (C<sub>1</sub>-C<sub>10</sub>)alkenyl, (C<sub>1</sub>-C<sub>10</sub>)alkynyl, (C<sub>1</sub>-C<sub>10</sub>)alkoxy or (C<sub>3</sub>-C<sub>8</sub>)cycloalkyl, wherein any alkyl, alkenyl, alkynyl, alkoxy or cycloalkyl may optionally be substituted with one or more halogen, OH, CN, NO<sub>2</sub>, trifluoromethyl, SR, or NRR, wherein each R is independently H or (C<sub>1</sub>-C<sub>10</sub>)alkyl;

R<sub>4</sub> is H, (C<sub>1</sub>-C<sub>10</sub>)alkyl, (C<sub>1</sub>-C<sub>10</sub>)alkenyl, (C<sub>1</sub>-C<sub>10</sub>)alkynyl, (C<sub>1</sub>-C<sub>10</sub>)alkoxy or (C<sub>3</sub>-C<sub>8</sub>)cycloalkyl, wherein any alkyl, alkenyl, alkynyl, alkoxy or cycloalkyl may optionally be substituted with one or more halogen, OH, CN, NO<sub>2</sub>, trifluoromethyl, SR, or NRR, wherein each R is independently H or (C<sub>1</sub>-C<sub>10</sub>)alkyl;

R<sub>5</sub> is H, halogen, (C<sub>1</sub>-C<sub>10</sub>)alkyl, (C<sub>1</sub>-C<sub>10</sub>)alkenyl, (C<sub>1</sub>-C<sub>10</sub>)alkynyl, (C<sub>1</sub>-C<sub>10</sub>)alkoxy, (C<sub>1</sub>-C<sub>10</sub>)alkanoyl, (=O), (=S), OH, SR, CN, NO<sub>2</sub> or trifluoromethyl, wherein any alkyl, alkenyl, alkynyl, alkoxy or alkanoyl may optionally be substituted with one or more halogen, OH, SH, CN, NO<sub>2</sub>, trifluoromethyl, NRR or SR, wherein each R is independently H or (C<sub>1</sub>-C<sub>10</sub>)alkyl; and

X is O, S or NR wherein R is H or (C<sub>1</sub>-C<sub>10</sub>)alkyl, or a pharmaceutically acceptable salt thereof.

48. (Withdrawn) The method of claim 47 wherein R<sub>1</sub> is halogen, CN, NO<sub>2</sub>, trifluoromethyl or OH.
49. (Withdrawn) The method of claim 47 wherein R<sub>1</sub> is OH.
50. (Withdrawn) The method of claim 47 wherein R<sub>2</sub> is (=O).
51. (Withdrawn) The method of claim 47 wherein R<sub>3</sub> is H or (C<sub>1</sub>-C<sub>10</sub>)alkyl.
52. (Withdrawn) The method of claim 47 wherein R<sub>3</sub> is methyl.
53. (Withdrawn) The method of claim 47 wherein R<sub>4</sub> is H or (C<sub>1</sub>-C<sub>10</sub>)alkyl.
54. (Withdrawn) The method of claim 47 wherein R<sub>4</sub> is H.

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55. (Withdrawn) The method of claim 47 wherein  $R_5$  is halogen, CN,  $NO_2$ , trifluoromethyl or OH.
56. (Withdrawn) The method of claim 47 wherein  $R_5$  is OH.
57. (Withdrawn) The method of claim 47 wherein X is O or S.
58. (Withdrawn) The method of claim 47 wherein X is O.
59. (Withdrawn) The method of claim 47 wherein both ----- are a single bond.
60. (Withdrawn) The method of claim 47 wherein one ----- is a double bond.
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61. (Withdrawn) The method of claim 47 wherein both ----- are a double bond.
62. (Withdrawn) The method of claim 45 wherein  $R_1$  is OH,  $R_2$  is (=O),  $R_3$  is methyl,  $R_4$  is H,  $R_5$  is OH, X is O, and both ----- are a double bond.

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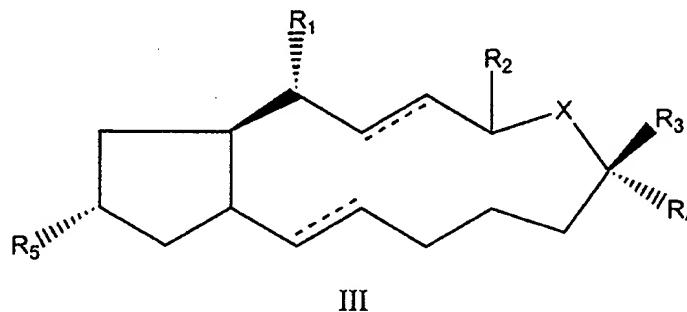
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63. (Withdrawn) The method of claim 47 wherein the compound is a compound of formula (III):



64. (Withdrawn) The method of claim 63 wherein  $R_1$  is halogen, CN,  $\text{NO}_2$ , trifluoromethyl or OH.
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65. (Withdrawn) The method of claim 63 wherein  $R_1$  is OH.
66. (Withdrawn) The method of claim 63 wherein  $R_2$  is (=O).
67. (Withdrawn) The method of claim 63 wherein  $R_3$  is H or  $(\text{C}_1\text{-C}_{10})$ alkyl.
68. (Withdrawn) The method of claim 63 wherein  $R_3$  is methyl.
69. (Withdrawn) The method of claim 63 wherein  $R_4$  is H or  $(\text{C}_1\text{-C}_{10})$ alkyl.
70. (Withdrawn) The method of claim 63 wherein  $R_4$  is H.
71. (Withdrawn) The method of claim 63 wherein  $R_5$  is halogen, CN,  $\text{NO}_2$ , trifluoromethyl or OH.
72. (Withdrawn) The method of claim 63 wherein  $R_5$  is OH.
73. (Withdrawn) The method of claim 63 wherein X is O or S.

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74. (Withdrawn) The method of claim 63 wherein X is O.
75. (Withdrawn) The method of claim 63 wherein both ----- are a single bond.
76. (Withdrawn) The method of claim 63 wherein one ----- is a double bond.
77. (Withdrawn) The method of claim 63 wherein both ----- are a double bond.
78. (Withdrawn) The method of claim 63 wherein R<sub>1</sub> is OH, R<sub>2</sub> is (=O), R<sub>3</sub> is methyl, R<sub>4</sub> is H, R<sub>5</sub> is OH, X is O, and both ----- are a double bond.

79. (Currently Amended) The method of claim 1 or 87 wherein the mammalian cell is contacted with an agent that inhibits the activation of ubiquitin, the transfer of ubiquitin to the ubiquitin carrier protein, ubiquitin ligase, or a combination thereof.
80. (Currently Amended) The method of claim 1 or 87 wherein the mammalian cell is contacted with an agent that inhibits ubiquitin ligase.
81. (Currently Amended) The method of claim 1 or 87 wherein the mammalian cell is contacted with an agent that is a compound of formula (IV):



wherein R is hydrogen, an amino acid, or a peptide, wherein the N-terminus amino acid can optionally be protected at the amino group with acetyl, acyl, trifluoroacetyl, or benzyloxycarbonyl; A is an amino acid or a direct bond; A<sub>1</sub> is an amino acid; and R<sub>1</sub> is hydroxy or an amino acid, wherein the C-terminus amino acid can optionally be protected at the carboxy group with (C<sub>1</sub>-C<sub>6</sub>)alkyl, phenyl, benzyl ester or amide (e.g., C(=O)NR<sub>2</sub>, ~~wherein each R is independently hydrogen or (C<sub>1</sub>-C<sub>6</sub>)alkyl~~); or a pharmaceutically acceptable salt thereof.



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82. (Withdrawn) The method of claim 81 wherein the agent is H-Leu-Ala-OH, H-His-Ala-OH, or a combination thereof.
83. (Currently Amended) The method of claim 1 or 87 further comprising ~~administering~~ contacting the mammalian cell with a second agent that enhances the activity of the agent contacted with the mammalian cell.
84. (Original) The method of claim 83 wherein the second agent is EGTA.
85. (Canceled)
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86. (Currently Amended) The method of claim 1 or 87 wherein the mammalian cell is contacted with an agent that alters endosomal processing.
87. (Currently Amended) A method to identify an agent that enhances AAV transduction of a mammalian cell, comprising:  
a) contacting a mammalian cell with one or more agents and AAV; and  
b) identifying at least one agent that when contacted with the mammalian cell enhances transduction after viral binding to the cell membrane and before second strand synthesis which yields an expressible form of the viral genome, wherein the agent which is identified enhances AAV transport to the nucleus of the mammalian cell.
88. (Currently Amended) A method to identify an agent that enhances AAV transduction of a mammalian cell comprising:  
a) providing a mammalian cell contacted with an agent and AAV; and  
b) identifying an agent that when contacted with the mammalian cell enhances internalized AAV transport to the nucleus of the contacted mammalian cell.
89. (Currently Amended) A method to identify an agent that enhances AAV transduction of a mammalian cell, comprising:

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- a) contacting a mammalian cell with one or more agents and AAV; and  
b) identifying at least one agent that when contacted with the mammalian cell enhances internalized AAV transport to the nucleus of the mammalian cell.
90. (New) The method of claim 88 or 89 wherein the mammalian cell is contacted with an agent that enhances endosomal processing.
91. (New) The method of claim 88 or 89 wherein the mammalian cell is contacted with an agent that is an endosomal protease inhibitor.
92. (New) The method of claim 88 or 89 wherein the mammalian cell is contacted with an agent that is a cysteine protease inhibitor.
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93. (New) The method of claim 88 or 89 wherein the mammalian cell is contacted with an agent that is a peptide or analog thereof.
94. (New) The method of claim 88 or 89 wherein the mammalian cell is contacted with an agent that inhibits the activation of ubiquitin, the transfer of ubiquitin to the ubiquitin carrier protein, ubiquitin ligase, or a combination thereof.
95. (New) The method of claim 81 wherein the amide comprises  $C(=O)NR_2$ , wherein each  $R_2$  is independently hydrogen or  $(C_1-C_6)$ alkyl.